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RESEARCH ARTICLE

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Hypoglycemic, hematologic and lipid profile effects of *Chromolaena odorata* ethanol leaf extract in alloxan induced diabetic rats

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ABSTRACT

This study investigated the hypoglycemic, hematologic and hypolipidemic potentials of Chromolaena odorata ethanol leaf extract (CELE) in alloxan induced diabetic rats.Adult rats were divided into 5 groups of 7 rats each. Group1 which comprised of normal rats received 0.2ml normal saline and served as the normal control, while groups 2-5 comprised of Alloxan induced diabetic rats. Group 2 received no treatment and served as the diabetic control. Group 3 was treated with a reference drug, Glibenclamide (5mg/kg) while groups 4 and 5 received 150 and 300mg/kg of CELE respectively. All administrations were done via the oral route and lasted for 21 days. Results obtained indicate that all doses of CELE significantly (P<0.05) lowered glucose levels in the diabetic rats with 300mg/kg lowering blood glucose from 311.80 ± 37.10 in diabetic rats to 105.72 ± 4.11 by the end of 21 days of treatment. The hypoglycemic effect of CELEcompared favorably with that of the reference drug used. Red blood cells (RBC) counts, packed cell volume (PCV),hemoglobin values were all significantly raised (P < .05) in treated rats, while the increased WBC value in diabetic rats was lowered. The levels of total cholesterol, triglycerides, low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) were significantly (P<0.05) decreased in the diabetic treated rats with increase in the levels of high density lipoprotein cholesterol (HDL-C). The results therefore suggest that CELE contains principles with hypoglycemic, hematologic and hypolipidemic properties and could be of value in the management of diabetic mellitus and associated anemia and lipid abnormalities.

Key Words: Glucose, Hypoglycemic, Hypolipidemic, *Chromolaena odorata*, Rats.

INTRODUCTION

The use of plant materials for medicinal purposes is an ancient practice which has become even more relevant in modern perspective. Cost, availability, accessibility and effectiveness are some reasons attributable to the widespread use of these natures gift. The fact that the tropics in which majority of Africa lies is host to a lot of medicinal plants accounts for the numerous plant based scientific works being carried out here, all in a bid to discover/develop strong agents which can be used to meet the numerous health challenges of man. No wonder [1], reported that the primary aim of sourcing for plants drug through any of the known strategies is mainly to detect the

active ingredients in plants that exert definite pharmacological effects in the body, since the results of such investigations would most often serve as a lead for the biological evaluation of these plants and to new drug discovery. *Chromolaena odorata*is one of such plants that are being investigated for diverse health benefits.*Chromolaena odorata* is a rapidly growing perennial herb, a multi -stemmed shrub up to 2.5m tall in open areas. It has soft stems but the base of the shrub is woody. In shady areas, it becomes etiolated and behaves as a creeper, growing on other vegetation. It can then become up to 10m tall. The plant is hairy and glandular and the leaves give off a pungent aromatic odour when crushed. The leaves are opposite, triangular to elliptical with serrated edges, 4-10 cm long by 1-5 cm wide. The plant can regenerate from the roots. In favorable conditions, the plant can grow more than 3cm a day [2].Available literature reveals that the plant contains carcinogenic pyrrolizidine alkaloid and can cause toxicity and allergic reactions in cattle [2]. However there is report that the extract from the leaves is used traditionally to treat skin wounds.

Diabetes mellitus (DM) is a common disease associated with increased morbidity and mortality and can be defined as a group of metabolic diseases characterized by chronic hyperglycemia, due to defective insulin secretion, insulin action or both, resulting in impaired carbohydrate, protein and lipid metabolism [4]. Among the pathophysiological anomalies associated with the condition are hyperglycemia and lipid profile abnormalities [4, 5] and Anemia [6, 7]. Treatment is based on oral hypoglycemic agents and insulin which in most cases have so many side effects. This study was designed to evaluate the hypoglycemic, hematologic and hypolipidemic potentials of *Chromolaena odorata* ethanol leaves extract (CELE) with a view to making these common weed more useful to man and to improve on the scanty literature available on the plant.

MATERIALS AND METHODS

2.1 Plant materials (Collection and Preparation)

Fresh leaves of *Chromolaena odorata*were collected from Umudike, Ikwuano Local Government Area of Abia State, Nigeria. The leaves were dried under shade in the laboratory for 7 days after which they were ground to powder using an electric blender. Thirty five (35) gramsof the powdered material was introduced into the extraction chamber of the soxhlet extractor and extraction was done using ethanol as solvent for 48 hours with temperature maintained at 70^0C. At the end of the period, the extract was dried in a laboratory oven at 40^0C to obtain a dried extract weighing 12.6g and represented a yield of 36%.

2.2 Animals

Adult albino rats of both sexes (150-180g) obtained from the Animal house of the University of Nigeria, Nsukka were used. They were fed with standard rat feed, with water ad libitum but starved for 12 hours prior to the commencement of experiment. All animal experiments were conducted in compliance with NIH guidelines for care and use of laboratory Animals (Pub. No.85-23, Revised, 1985, as expressed by [4]. The study was conducted at the Physiology Laboratory of the Department of Veterinary Physiology, Pharmacology, Biochemistry and Animal Health, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

2.3 Acute toxicity study (LD_{50})

Thirty five mice of both sexes weighing 20-25g were divided into 7 groups of 5 mice each and were assigned graded oral doses of CELE in the order 500,1000,2000,2500,3000,4000 and 5000mg/kg body weight. The mice were kept in aluminum cages and allowed free access to feed and water. Observation was made for toxicity signs and number of deaths within a period of 24 hours. LD_{50} value was then determined using the method of Karber, as expressed by Enegide *etal.*, (2013) [8].

2.4 Induction of Diabetes

Diabetes was induced in rats by a single intraperitoneal (I.P) injection of freshly prepared solution of Alloxan monohydrate (160mg/kg). Eight days later rats with blood glucose concentration above 190mg/dl were considered diabetic and 28 of these diabetic rats and 5 normal ones were used for the study.

2.5Blood glucose level, hematological and lipid profile studies

While the 7 normal rats were placed in group 1 to serve as the normal control, 28 diabetic oneswere divided randomly into 4 groups(2-5) of 7 rats each. Group 2 which served as the diabetic control received no treatment. Group 3 was treated with a reference drug (Glibenclamide, 5mg/kg body weight), while groups 4 and 5 received 150 and 300mg/kg of CELE respectively. All treatments were done daily via the oral route and lasted 21 days.

2.6Acute and sub-acute effect of CELEon blood glucose levels

On the first day of treatment blood was obtained from the tail of each rat in all groups (1-5) by tail snip method prior to and at 2 and 5 hours following treatment and glucose levels were determined for each rat using a glucose meter following standard procedures prescribed by the producer, Roche diagnostic Company, Germany. For the sub-acute studies, the tests were repeated on day 7, 14 and 21.

2.7 Hematological Studies

All rats were sacrificed on the 22nd day and blood was collected by cardiac puncture into EDTA bottles to be used for the determination of hematological parameters including: Red blood cell (RBC) counts, Packed cell volumes (PCV), Hemoglobin (Hb) Concentrations, White blood cell (WBC) counts, White blood cell differential counts, Platelets counts, Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC). These parameters were obtained at once for each blood sample using an Automated Hematology Analyzer–MC-2800 (Mindray Company, China).

2.8Lipid Profile Studies

A portion of each blood sample was centrifuged to obtain a clear plasma which was used to estimate total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and triglycerides (TG) using commercial kits and following standard procedures outlined by the producer, Randox Laboratories, UK.

2.9 Statistical Analysis

Results were expressed as mean ± SEM.Statistical analysis was performed by one-way analysis of variance (ANOVA), students t-test at 95% level of significance was used to assess significant difference between controls and treated group.

RESULTS

3.1 Acute toxicity

Seven groups of 5 rats each were administered varying doses of CELE. Deaths were recorded in some groups within the 24 hours period of acute of the acute toxicity study and an LD50 value of 2700mg/kg body weight was obtained.

Table 1: Acute effect of CELE on blood glucose level in diabetic rats

		0HR	2HRS	5HRS
Group	**Treatment**	**Glucoselevel (mg/dL)**	**Glucose level mg/dL**	**Glucose level mg/dL**
1	Normal control	110.40 ±7.10	107.80 ±4.52	101.40 ±3.90
2	Diabetic control	311.80 ±37.10	307.80 ±34.30	311.60 ±51.40
3	Glibenclamide 5mg/kg	288.20 ±37.50*	206.00 ±37.00*	150.60 ±10.50*
4	CELE, 150mg/kg	232.20±11.10*	227.20 ±14.40*	154.40 ±13.50*
5	CELE, 300mg/kg	223.40 ±13.30*	201.80 ±8.87*	164.80 ± 12.20*

**P<.05 versus diabetic control*

Table 2: Sub-acute effect of CELE on blood glucose level in diabetic Rats

		Day 7	Day 14	Day 21
Group	**Treatment**	**Glucose level in mg/Dl**	**Glucose level inmg/dL**	**Glucose level in mg/dL**
1	Normal control	98.40 ±4.69	102.00 ±2.29	103.40 ±2.29
2	Diabetic control	377.40 ±56.30	323.20 ±27.87	318.00 ±25.30
3	Glibenclamide 5mg/kg	104.40 ±6.34*	80.80 ±5.21*	82.13±4.80*
4	CELE, 150mg/kg	121.40 ±2.50	95.20 ±8.13*	96.10±3.82*
5	CELE, 300mg/kg	110.80 ±5.00*	90.60 ±5.21*	105.72±4.11*

**P<.05 versus diabetic control*

3.2 Acute and sub-acute effects of CELE on blood glucose levels in diabetic rats

All doses of CELE significantly (P<0.05) lowered blood glucose levels in the diabetic treated rats within the 5 hours of the acute study. By the end of the period 150 and 300mg/kg of CELE had reduced glucose levels in diabetic treated rats 232.20 ±11.10 and 223.40± 13.3 to 154.40± 13.50 and 164.80± 12.20 respectively. (Table 1). By the end of the 21 days of sub-acute study the glucose levels of all diabetic rats treated with CELE returned to about normal

values.The blood glucose levels of diabetic rats treated with CELE was significantly (P<0.05) different from that of the diabetic control rats but compared favorably with that of diabetic rats treated with Glibenclamide (Table 2).

3.2 Effects of CELE on Hematological Parameters

All doses of CELE significantly (P<0.05) increased RBC, PCV, and HB values (Table 3) and lowered WBC counts in the diabetic treated rats.MCH, Platelets, Lymphocytes were raised to about normal values, whileMCV, MCHC and Midcells (monocytes, eosinophils and basophils) were not significantly affected. (Tables 3 and 4).

Table 3: Effect of CELE on RBC, PVC, HB, MCV, MCH and MCHC in diabetic Rats

Group	Treatment	RBC x 10^{12} per liter	PCV(%)	HB (g/dL)	MCV (fL)	MCH (pg)	MCHC(g/dL)
1	Normal control	6.80±0.26	40.97±2.43	12.4±0.53	60.23±3.67	18.23±0.29	30.50±1.71
2	Diabetic control	4.77±0.17	30.10 ±1.81	10.03±0.31	63.40 ±3.96	21.03±1.14	33.50±2.20
3	Glibenclamide,5mg/kg	5.10±0.25	30.97±1.61	10.30±0.26	60.73±4.32	20.20±0.18	33.26±1.05
4	CELE, 150mg/kg	6.89±0.27*	36.50±2.13*	12.38±0.39*	53.03±1.10*	17.98±0.37*	33.88±0.47
5	CELE, 300mg/kg	6.08±0.26*	31.87±1.32*	11.13±0.49*	52.37±0.27*	18.30±0.50*	33.26±1.05

**P*<.05 versus diabetic control

TABLE 4:Effects of CELE on platelet counts, WBCand differential WBC Counts in diabetic rats

Group	Treatment	Platelets x 10^9/L	WBC x 10^9/L	Lymphocytes %	Neutrophils %	Midcells (Eosinophils, Monocytes& Basophils) %
1	Normal control	909 ± 227	9.76±2.97	46.57±5.93	33.87±7.98	19.70±1.63
2	Diabetic control	611 ± 102	29.63±2.29	51.31±0.57	27.43±2.06	21.57±2.03
3	Glibenclamide, 5mg/kg	571 ± 103	17.7±0.44*	46.97±1.88*	29.77±0.24*	23.20±1.68
4	CELE, 150mg/kg	1087.30 ± 265.50	10.43±3.31*	48.23±5.48	25.25±5.71	21.98±1.17
5	CELE, 300mg/kg	909± 32.96	12.20±3.29*	40.80±6.01*	35.70.50±3.95*	23.50±0.71

** P< .05 versus diabetic control*

3.3 Effect of CELE on lipid profile in diabetic rats

The elevated total cholesterol, triglycerides, LDL-C and VLDL-C in diabetic rats were significantly (P< .05) lowered by all doses of CELE while the lowered HDL-C was raised, and tilted towards normal values (Table 5).

TABLE 5:Effect of CELE onlipid profile in diabetic rats

Group	Treatment	Total Cholesterol mg/dL	Triglycerides mg/dL	HDL-C mg/dL	LDL-C mg/dL	VLDL-C mg/dL
1	Normal control	81.29±0.32	37.97±0.64	40.26±0.73	33.44±0.84	7.59±0.13
2	Diabetic Control	158.10±3.27	70.83±0.82	17.05±0.53	126.90 ±3.43	14.17±0.18
3	Glibenclamide 5mg/kg	52.10±1.69*	41.68±0.42*	35.32±0.51*	8.44±1.97*	8.34±0.08*
4	CELE, 150mg/kg	70.50±2.43*	53.59±1.52*	39.73±0.74*	20.05±20.59*	10.72±0.30*
5	CELE, 300mg/kg	55.12±3.44*	48.77±0.50*	25.88±0.65*	19.49±3.30*	9.75±0.10*

** P<.05 versus diabetic control*

DISCUSSION

The toxicity observed during the 24 hours acute toxicity study of CELE (LD_{50} = 2700 mg/kg) suggests that the extracts could be toxic at doses beyond effective limits. The level of toxicity observed may be due to the presence of toxic phytochemical components in the plant. Carcinogenic pyrrolizidine alkaloids present in *Chromolaena odorata* leaf extract [2]andthe relative abundance of toxic substances including alkaloids, lactones, tannins, saponins and steroids [9], may have accounted for the level of toxicity observed during the acute toxicity study of the extract. However at low doses as used in this work, CELE was found to be well tolerated and a potent medication.

Alloxan monohydrate successfully induced hyperglycemia in the rats used. Hyperglycemia is usually the first sign in the development of diabetes mellitus. Alloxan monohydrate achieved this effect by selectively destroying the pancreaticbeta cells of the islets of langerhans in the rats. This marked degeneration of the islets lowered insulin secretion with reduction in the rate of conversion of glucose to glycogen, the result of which is the marked increase

of sugar levels in the diabetic rats.[10] had reported that Alloxan induces diabetes by destroying the beta cells of the pancreas which are involved in the synthesis, storage and release of insulin, the peptide hormone regulating carbohydrate and lipid metabolism leading to high blood sugar levels. This high sugar levels confirms the development of diabetes mellitus [5].

All doses of CELE significantly (P<.05) lowered blood glucose levels within the acute and sub-acute periods of study returning the blood glucose levels in diabetic rats to about normal values by the 21 days treatment period. This result suggests that CELE contain substances with hypoglycemic properties and tends to agree with [11], who reported that the hydro-ethanolic extract of *Chromolaena odorata* lowered glucose levels when administered to rats. This hypoglycemic effect may have been achieved by increasing insulin selection and peripheral utilization of glucose in diabetic rats, inhibition of endogenous glucose production, inhibition of intestinal glucose absorption and/or regenerating existing beta cells. These mechanisms have all been reported to be responsible for lowering blood sugar levels [10, 12, 13].

Anemia was found to accompany the development of diabetes mellitus as the diabetic control rats all had significantly (p< .05) lowered RBC, PCV, HB, MCH, MCV and MCHC values when compared to the normal control rats. The results agree with [6, 7, and 14], who reported that in diabetes mellitus, there is the development of anemia, particularly, the hypochromic type, due to fall in the iron content of the body resulting from oxidation stress associated with the condition. All doses of CELE restored the values of these parameters to normal in the diabetic treated rats after days of treatment. This anti-anemic activity of CELE may be attributedto the high iron content of its chlorophyll, as seen in other green, leafy vegetables[7] and/or the ability to improve bone marrow functions [10].

In the lipid profile studies, the elevated total cholesterol, triglycerides, low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) with decreased high density lipoprotein cholesterol (HDL-C) observed in the diabetic control group also suggest that the development of diabetes mellitus is usually accompanied by anomalies in body lipid composition. This finding agrees with existing literature report that the development of diabetes mellitus is usually followed by marked increase in blood cholesterol, triglycerides, LDL-C, VLDL-C and a reduction in HDL-C [4].The lowering of cholesterol, triglycerides, LDL-C and VLDL-C and increase inHDL-C observed in the rats treated with 150 and 300mg/kg of CELE indicates presence of principles with hypolipidemic properties in the extract. Prasad et al., 2005, [9],had reported the presence of saponin and flavonoids in the leaf extract of *Chromolaena odorata,* both of which have been implicated in the lowering of blood cholesterol [15].

CONCLUSION

The results obtained from this study indicate that *Chromolaena odorata* leaves contain substances with hypoglycemic properties and at low doses could be a safe and potent agent to be employed in the treatment of diabetes mellitus and resultinghematological and lipid profile anomalies.

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